



## Original Article

# Exhaled carbon monoxide levels in preschool-age children with episodic asthma

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**Abstract** **Background:** The concentration of exhaled carbon monoxide (eCO) in young children with stable asthma and during acute asthma attack is not known.

**Methods:** A sampling bag was developed to collect the exhaled air of preschool children. A total of 257 preschool-age children ( $\geq 3$  years and  $\leq 6$  years old) were studied; 111 had a diagnosis of asthma (43 suffering a mild asthma attack and 68 without active asthmatic symptom), 99 had upper respiratory infection (URI) and 47 were healthy.

**Results:** In preschool-age children, eCO levels of those with asthma attacks (mean  $\pm$  SE,  $2.7 \pm 0.3$  p.p.m.,  $n = 43$ ) were significantly higher than those of subjects with asymptomatic asthma ( $0.5 \pm 0.1$  p.p.m.,  $P < 0.05$ ), URI ( $0.8 \pm 0.1$  p.p.m.,  $P < 0.05$ ) and healthy children ( $0.4 \pm 0.1$  p.p.m.,  $P < 0.05$ ). A multivariate linear regression model showed that eCO was higher in children with asthma attacks independent of age and gender. In 33 asthmatic children followed before and after treatment, eCO levels during asthma attacks significantly decreased after inhalation therapy with a combination of salbutamol and sodium cromoglycate (before therapy,  $2.9 \pm 0.4$  p.p.m.; after therapy,  $0.6 \pm 0.1$  p.p.m.,  $P < 0.0001$ ).

**Conclusions:** The measurement of eCO using a novel collecting system is useful in the recognition of asthma in preschool children.

**Key words** asthma, breath test, carbon monoxide, child, preschool, respiratory therapy.

Asthma is a chronic lung disorder characterized by airway inflammation.<sup>1</sup> The inducible form of heme oxygenase (HO)-1 is expressed in airway epithelial cells,<sup>2</sup> endothelial cells,<sup>3</sup> and alveolar macrophages.<sup>4</sup> Carbon monoxide (CO) is produced endogenously by HO, and is known to be present in a low concentration in the exhaled air of normal subjects.<sup>5,6</sup> Upregulation of HO-1 by oxidative stress and pro-inflammatory cytokines in the airways and lung parenchyma increases the levels of exhaled CO (eCO) in patients with the inflammatory pulmonary diseases such as bronchiectasis, upper respiratory tract infection (URI), acute pneumonia, silicosis and seasonal allergic rhinitis.<sup>4,7–12</sup> Recently, eCO levels were shown to be elevated in adults<sup>6,13</sup> and school-age children with persistent asthma.<sup>14</sup> In early childhood, however, measuring the eCO concentrations of episodic asthma in acute asthma attacks or in stable conditions and for other respiratory diseases is not feasible using the conventional method.<sup>15</sup>

We developed a simple sampling system, which allows the measurement of eCO concentration in preschool-age children with asthma with or without attacks or those with URI. We

examined the hypothesis that eCO reflects disease status and treatment effects in young children.

## Methods

### Study design and subjects

Preschool-age children ( $\geq 3$  years and  $\leq 6$  years old) with asthma with or without attacks, those with URI and healthy children were recruited at Ohara Children's Clinic, Fukushima from June 2006 to June 2008. We enrolled only children with mild asthma attack classified according to the Japanese Pediatric Guideline for the Treatment and Management of Bronchial Asthma (JPGL) 2005.<sup>16</sup> The criteria of mild asthma attack were breathless wheezing (+/-), retraction (-)-(+), prolongation of expiration (-), lying down posture, cyanosis (-) and  $SpO_2 \geq 96\%$ . Intermittent asthma was defined as occurrence of symptoms fewer than several times a year and only brief exacerbations.<sup>16</sup> We measured serum IgE levels in asthmatic subjects using a commercially available method. We followed these children to control their symptoms. We defined asymptomatic asthma as a history of infrequent mild asthma without exacerbations for at least 8 weeks before the study. All asthma patients did not have any respiratory history other than asthma. We excluded children from the study whose parents were current smokers. Twenty school-age children were recruited to confirm the correspondence of indirect and direct

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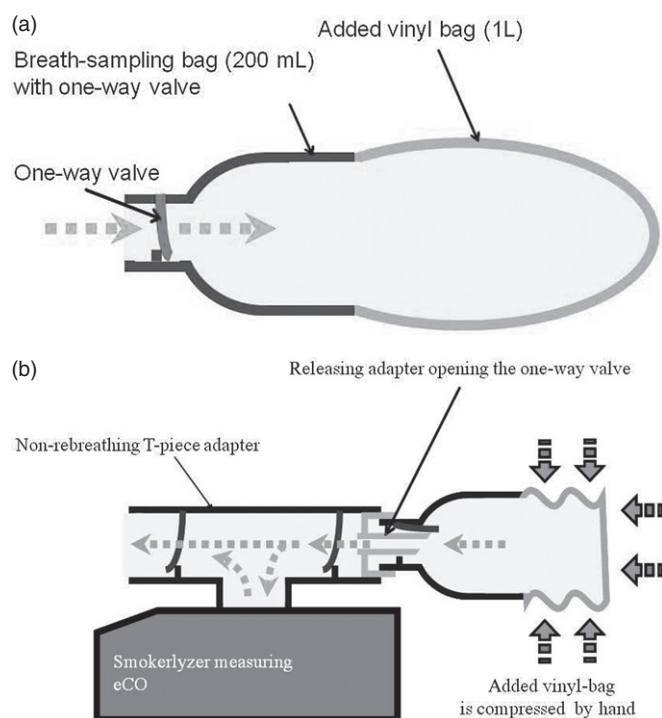


**Fig. 1** Kit to sample exhaled air and measure exhaled carbon monoxide (eCO) in preschool-age children. (a) Schematic diagram of a simple sampling bag. (b) Measurement of eCO in the sampling bag.

method of eCO measurements. This study was conducted in accordance with the Declaration of Helsinki, and was approved by the institutional review board at Tohoku University Graduate School of Medicine. Informed parental consent was obtained orally.

#### **Collection of exhaled air and eCO measurements in preschool-age children**

We developed a simple kit to sample exhaled air and measure CO in preschool-age children. It consists of a breath-sampling bag with one-way valve, a releasing adapter, a T-adapter with non-rebreathing valve and a Micro Smokerlyzer (Bedfont Scientific, Harrietsham, UK; Fig. 1). We used a breath-sampling bag (200 mL) with a one-way valve, which is usually used for the  $^{13}\text{C}$  urea exhaled test (Otsuka Pharmaceutical, Tokyo, Japan). To sample sufficient volume of exhaled air for the measurement with a Micro Smokerlyzer, we cut the bottom of the bag and connected it with a polyvinyl bag (18 cm  $\times$  27 cm  $\times$  2 cm, approx. 1 L volume; Fig. 1a). Preschool-age children blew into the sampling bag (Fig. 2). After collecting exhaled air, we connected the sampling bag to the Micro Smokerlyzer using the T-adapter with non-rebreathing valve and pushed out the air into the instrument manually to measure eCO concentration (Fig. 1b). The measurement range of the Micro Smokerlyzer is 0–1000 p.p.m. by volume with a resolution of 1 p.p.m.<sup>14,17,18</sup> The instrument was calibrated with a gaseous mixture containing 50 p.p.m. CO according to the manufacturer's instructions. The CO concentration in exhaled air measured by this instrument is

reported to correlate closely with blood carboxy-hemoglobin concentration over the range of values encountered in smokers and in non-smokers.<sup>6,10</sup> We compared our indirect sampling-bag method (offline method) with the direct measurement by Micro Smokerlyzer (online method) in 20 school-age children. We confirmed the background CO level in the clinic using Fluke CO-220 Carbon Monoxide Meter (Everett, WA, USA) to be 0 p.p.m. We measured eCO levels of the patients' mothers (or lodgers) to check the environmental CO of their home and confirmed this to be in the normal range (0–1 p.p.m.). We analyzed eCO on single measurements. Reliability of the sample bag method for 14 children and two eCO measurements on the same occasion was satisfactory (intraclass correlation coefficient, 0.79).

We also measured eCO concentrations both before and several days after the treatment of asthma in some of the asthmatic preschool-age children. Patients were treated with the combined inhalation therapy with salbutamol and sodium cromoglycate (SCG) according to the guideline for the treatment of mild asthma attack.<sup>16,19</sup> The combination therapy consisted of a mixture of either 0.1 mL (<1 year of age), 0.2 mL (1–5 years of age) or 0.3 mL (>6 years of age) of 5 mg/mL aqueous solution of salbutamol and 2 mL of 1% aqueous solution of SCG, which was delivered for 3 min using a jet nebulizer.<sup>19</sup> SCG + salbutamol inhalation therapy was repeated a maximum of four times per day, if the symptom persisted. Long-term asthma management was performed following the JPGL 2005 guideline.<sup>16</sup>

We also measured arterial oxygen saturation using pulse oxymetry ( $\text{SpO}_2$ ) with a pulse oxymeter<sup>10,20</sup> simultaneously with measurement of eCO concentration to monitor respiratory conditions in patients with an acute asthma attack. We excluded subjects from the study whose respiratory conditions prevented complete filling of the sampling bags.

#### **Statistical analysis**

Numerical data are presented as mean  $\pm$  SE. Numerical data were compared among groups using ANOVA with Tukey's ad hoc test



**Fig. 2** Measurement of exhaled carbon monoxide in a preschool-age child.

**Table 1** Subject characteristics

|                      | Healthy    | Asymptomatic asthma        | URI       | Asthma attack             | <i>P</i> |
|----------------------|------------|----------------------------|-----------|---------------------------|----------|
| <i>n</i>             | 47         | 68                         | 99        | 43                        |          |
| Age (years)          | 5.3 ± 0.2* | 4.5 ± 0.1                  | 4.3 ± 0.1 | 4.6 ± 0.2                 | <0.01    |
| Female, <i>n</i> (%) | 24 (51)    | 31 (46)                    | 55 (56)   | 12 (28)                   | 0.02     |
| IgE (IU/mL)          | ND         | 371 ± 107 ( <i>n</i> = 23) | ND        | 468 ± 92 ( <i>n</i> = 30) | 0.9      |
| eCO (p.p.m.)         | 0.4 ± 0.1  | 0.5 ± 0.1                  | 0.8 ± 0.1 | 2.7 ± 0.3 <sup>#</sup>    | <0.01    |
| SpO <sub>2</sub> (%) | ND         | ND                         | ND        | 97.0 ± 0.2                | ND       |

ANOVA or unpaired *t*-test or chi-square test, as appropriate. \**P* < 0.05 vs asymptomatic asthma, URI and symptomatic attacks using Tukey's ad hoc test. <sup>#</sup>*P* < 0.05 vs healthy, asymptomatic asthma and URI using Tukey's ad hoc test. eCO, exhaled carbon monoxide; ND, not done; SpO<sub>2</sub>, arterial oxygen saturation by pulse oxymetry; URI, upper respiratory infection.

or unpaired *t*-test, as appropriate. Categorical data were compared among groups using chi-squared test or Fisher's exact test, as appropriate. Changes between eCO during asthma attacks and that after treatment were analyzed using paired *t*-test. *P* < 0.05 was considered as significant. To evaluate the influence of age and gender on eCO, a multivariate linear regression model was generated with age, gender (male = 1) and disease status (asthma attack = 1) as independent variables.

## Results

### Indirect sampling bag method and direct measurement

We directly compared eCO measured with our indirect sampling bag method (offline method) and those measured with the conventional direct method (online method) in 20 school-age children. The two methods were well correlated (*r* = 0.83, *P* < 0.0001) and the bias (offline method – online method) was within a satisfactory range (bias ± SD = −0.2 ± 0.6 p.p.m.). eCO levels ranged between 0 and 3 p.p.m. in this subject group.

### eCO in preschool-age children

We enrolled 111 preschool-age children with asthma, in whom 43 had mild asthma attacks and 68 were asymptomatic. We also enrolled 99 children with URI and 47 healthy children in the same age range (Table 1), but healthy children were older than those in the other groups. Male children dominated in the subjects with asthma attacks. The serum IgE levels were elevated both in the asymptomatic and symptomatic asthma children (371 ± 107 IU/L, 468 ± 92 IU/L, respectively), which indicated that the present asthmatic participants had mild atopic asthma (Table 1). The SpO<sub>2</sub> level was 97.0 ± 0.2%, which indicated that the respiratory state of the present patients was not severely depressed (Table 1). Treatment at the time of eCO measurement was not different between asymptomatic asthma and children with attacks (Table 2). Thirteen out of 43 children with asthma attacks had preceding history of acute bronchiolitis (influenza A infection, *n* = 1; mycoplasma infection, *n* = 2; respiratory syncytial virus infection, *n* = 1; and common cold, *n* = 9). The remaining 30 children had no preceding symptoms.

The eCO level during asthma attacks in preschool-age asthmatic children (2.7 ± 0.3 p.p.m.) was significantly higher compared to that in healthy preschool-age children (0.4 ± 0.1 p.p.m., *P* < 0.05), that in asymptomatic preschool-age asthmatic children (0.5 ± 0.1 p.p.m., *P* < 0.05) and that in children with URI (0.8 ±

0.1 p.p.m., *P* < 0.05; Table 1; Fig. 3). There were no significant differences, however, in eCO concentration between healthy children, children with episodic asthma without current exacerbations and children with URI.

Male children tended to have higher eCO levels than female children in the subgroups (Table 3). Multivariate linear regression model however, showed that eCO was higher in children with asthma attacks independent of age and gender (Table 4).

In 33 preschool-age patients, the elevated eCO level during asthma attacks (2.9 ± 0.4 p.p.m.) significantly decreased after inhalation therapy (0.6 ± 0.1 p.p.m., *P* < 0.0001; Fig. 4).

## Discussion

Using a novel system for sampling and measuring CO concentration of exhaled air of preschool-age children, we found that the eCO concentration was increased in young children with acute symptomatic asthma compared with healthy children, those with stable asthma and those with URI. Successful treatment of preschool-age children with an acute asthma attack resulted in a fall in the eCO concentration.

Previous reports showed that airway inflammation leads to an increased expression of HO-1 in the airway, resulting in an elevation of eCO in adult asthma patients<sup>6,13</sup> and in school-age children with chronic persistent asthma.<sup>14</sup> Few reports, however, describe airway pathophysiology during asthma attacks in preschool-age children with mild episodic asthma because of the difficulty in getting young children to cooperate with conventional methods of measuring eCO.<sup>15,17,20</sup> To overcome this problem, we developed a novel method of measuring eCO for children in this age group. The result of preliminary comparison between eCO

**Table 2** Baseline treatment at eCO measurement

|                     | Asymptomatic asthma<br><i>n</i> (%) | Asthma attack<br><i>n</i> (%) | <i>P</i> |
|---------------------|-------------------------------------|-------------------------------|----------|
| <i>n</i>            | 68                                  | 43                            |          |
| DSCG                | 24 (35)                             | 9 (21)                        | 0.14     |
| LTRA                | 10 (15)                             | 6 (14)                        | 1.00     |
| ICS                 | 7 (10)                              | 2 (5)                         | 0.48     |
| Anti-allergic drugs | 2 (3)                               | 0 (0)                         | 0.52     |
| No drug             | 41 (60)                             | 32 (74)                       | 0.15     |

DSCG, disodium cromoglycate; eCO, exhaled carbon monoxide; ICS, inhaled corticosteroids; LTRA, oral leukotriene receptor antagonists.

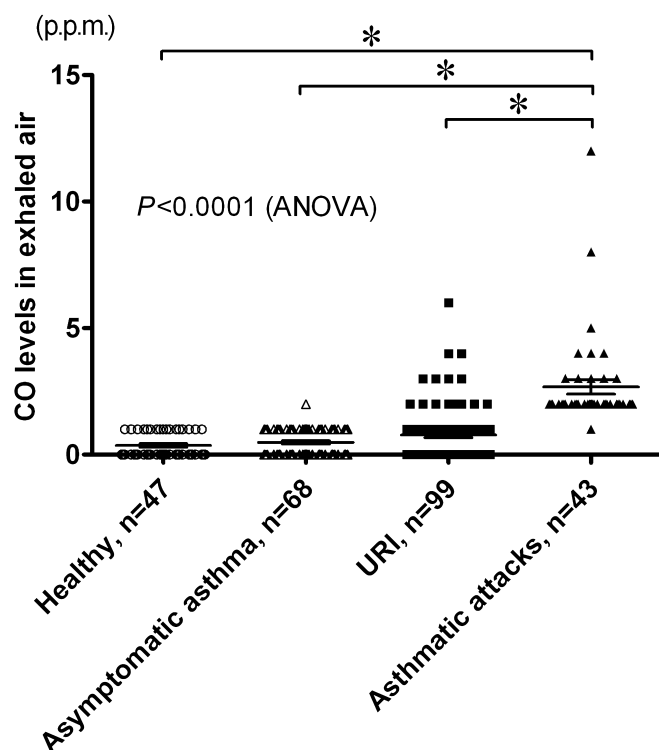


**Fig. 3** Exhaled carbon monoxide (CO) levels in preschool-age children (3–6 years of age). URI, upper respiratory infection. Horizontal bars, mean  $\pm$  SE. \* $P < 0.05$  compared with symptomatic attacks using Tukey's post-hoc test.

measured with our indirect sampling bag method (offline method) and that measured directly by Micro Smokerlyzer (online method) in 20 school-aged children was satisfactory. A previous report on fraction of exhaled nitric oxide showed that neither expiratory flow rate nor collection technique (online vs offline) significantly altered its capacity to discriminate asthma patients and healthy people,<sup>21</sup> which would support the present indirect sampling bag method for eCO.

The great advantage of monitoring exhaled gas such as CO is that the measurement procedure is completely non-invasive and can be performed repeatedly in children in whom more invasive techniques are not possible. Recently the American Academy of Pediatrics recommended the measurement of end-tidal CO as the only test that provides a direct measurement of heme catabolism and bilirubin production rate.<sup>22</sup> Although measurement of end-tidal CO was used in prematurely born infants, they had to use a

**Table 3** eCO vs gender and group

|                     | Female   |               | Male     |               | <i>P</i> |
|---------------------|----------|---------------|----------|---------------|----------|
|                     | <i>n</i> | Mean $\pm$ SE | <i>n</i> | Mean $\pm$ SE |          |
| Healthy             | 24       | 0.3 $\pm$ 0.1 | 23       | 0.5 $\pm$ 0.1 | 0.11     |
| Asymptomatic asthma | 31       | 0.4 $\pm$ 0.1 | 37       | 0.5 $\pm$ 0.1 | 0.35     |
| URI                 | 55       | 0.7 $\pm$ 0.1 | 44       | 0.9 $\pm$ 0.2 | 0.27     |
| Symptomatic attacks | 12       | 2.3 $\pm$ 0.3 | 31       | 2.8 $\pm$ 0.4 | 0.35     |

eCO, exhaled carbon monoxide; URI, upper respiratory infection.

**Table 4** Multivariate linear regression model to predict eCO

|                                    | $\beta$ | <i>P</i> |
|------------------------------------|---------|----------|
| Age                                | <0.01   | 0.98     |
| Gender (male = 1)                  | 0.09    | 0.09     |
| Disease status (asthma attack = 1) | 0.57    | <0.01    |

Multiple  $R^2 = 0.36$ ,  $P < 0.01$ .  $\beta$ , standardized regression coefficient; eCO, exhaled carbon monoxide.

special machine, CO-Stat End-Tidal Breath Analyzer (Natus Medical Inc.) for the measurement.<sup>22,23</sup> The strengths of the present method are not only its non-invasiveness but also its availability.

In preschool-age children, the elevated eCO concentrations during mild asthma attacks were significantly decreased after inhalation therapy with a  $\beta_2$ -agonist and SCG in episodic asthma. This suggests that monitoring of eCO may be useful for evaluating the responsiveness to the treatment in this age group. The exact mechanism of the decline in eCO levels after inhalation therapy, however, remains unclear. We treated the early childhood asthma with a combination therapy with salbutamol and SCG as recommended.<sup>19</sup> Because salbutamol acts as a potent bronchodilator,<sup>19</sup> the decrease in eCO concentrations might be due to an increase in airflow in this therapy. Furthermore, SCG was reported to have not only anti-inflammatory actions but also potentiating actions of the smooth muscle-relaxing properties of salbutamol in the airway.<sup>19</sup> Silkoff *et al.* reported a well-documented flow dependency of NO concentrations in exhaled air.<sup>24</sup> We previously performed expiratory peak flow measurement during mild asthma attacks in four patients (>8-year-old schoolchildren) and found a negative correlation between peak flow rate and eCO concentration.<sup>17</sup> We therefore speculate that the higher airflow could be one of the main reasons for decrease in the eCO levels after inhalation therapy with salbutamol and

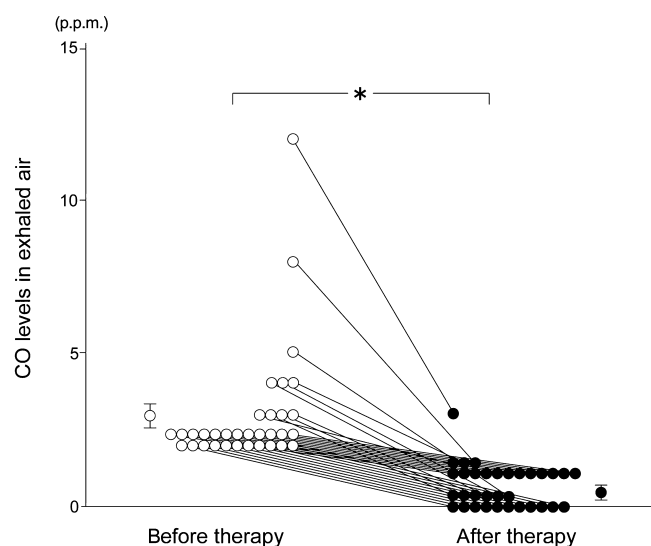


**Fig. 4** Exhaled carbon monoxide (CO) levels in preschool-age asthma attacks before and after inhalation therapy. Horizontal bars, mean  $\pm$  SE. \* $P < 0.0001$ .

SCG. It remains controversial, however, whether there is a simple flow dependency in the eCO concentration in patients with airway obstruction.<sup>25,26</sup>

A major disadvantage of eCO measurement to evaluate asthma is that environmental CO may affect the result of the eCO measurement. In the present study we confirmed the eCO of the patients' mothers (or lodgers) to be in the normal range (0–1 p.p.m.). We also confirmed that the environmental CO concentration in the clinic was 0 p.p.m. before eCO measurement. We excluded children from the study whose parents were current smokers to avoid the influence of smoking on eCO concentration. We must keep in mind the influence of environmental CO when we interpret eCO concentration in clinical practice. The eCO measurements, however, sometimes detect CO intoxication, which often mimics asthma or other respiratory disease, which might be a potential benefit of this method.<sup>27</sup>

The present study was an observational study with potential limitations. The preschool-age healthy controls were older than those in the other groups. The low eCO level, however, in the asymptomatic asthmatic preschool-age children in an age range similar to the symptomatic asthmatic children supports the assumption that the eCO levels of healthy children younger than the present controls should also be low. Male children dominated in the group with asthma attacks, and male children tended to have higher eCO levels than female children (Tables 1,3). Multivariate linear regression analysis, however, showed that eCO was elevated in children with asthma attack independent of age and gender (Table 4).

In conclusion, the measurement of eCO by a novel method is useful to diagnose asthma in preschool-age children. Sequential monitoring of exhaled CO by this method may provide complementary data for monitoring the control of asthma in preschool-age children. Further studies using this new system in a larger cohort to confirm the usefulness of eCO measurement to discriminate asthma from other diseases in this age group are warranted.

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